# NAMRU-2

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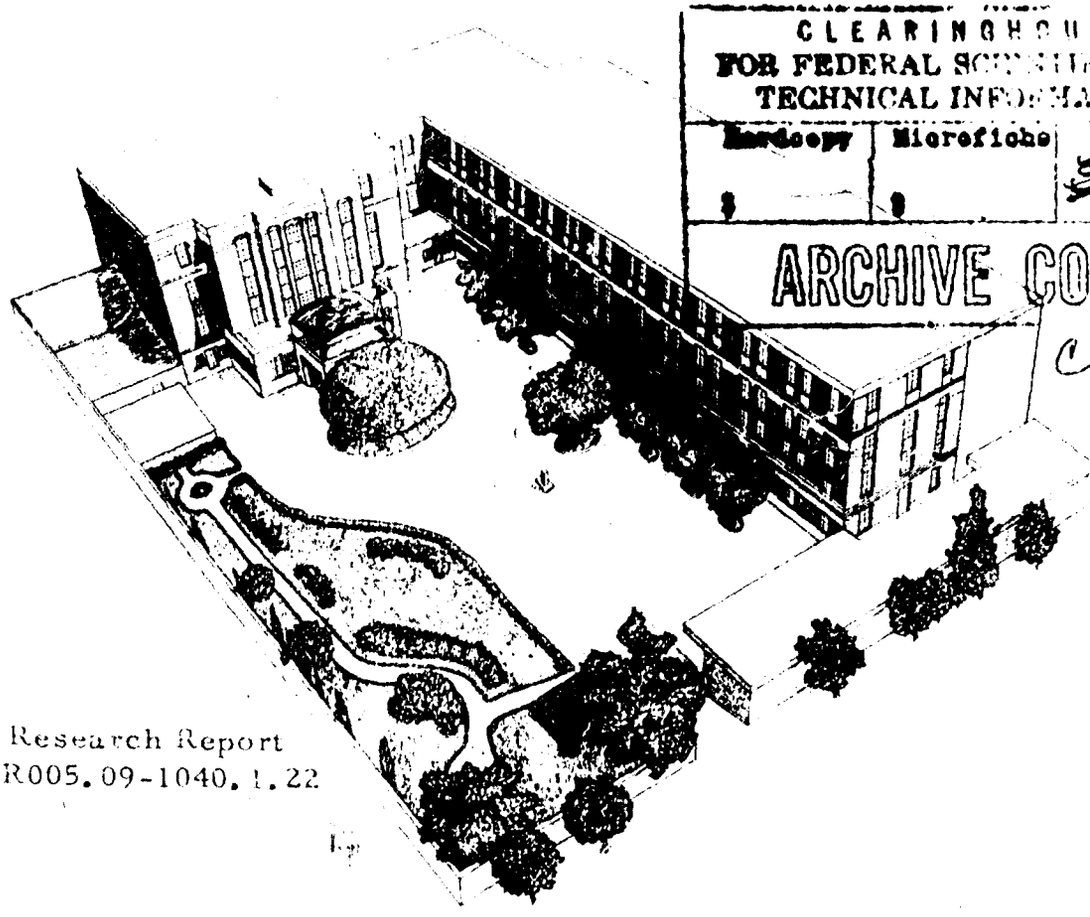

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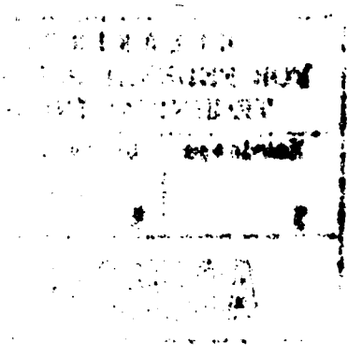

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# Ammonium Toxicity, a Potential Artifact in Preparation of Toxins[1]

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In this laboratory a number of experimental models are currently employed in an effort to gain some insight into the mechanism of the pathogenicity of cholera. These include the short-circuited frogskin, the everted intestinal (ileum) sac of rabbit, and the intestinal loop *in situ*.

Jenkin and Rowley (J. Gen. Microbiol. 21: 191, 1959) described a technique for preparation of a toxin from *Vibrio cholerae* by use of ammonium sulfate fractionation of sonicated preparations of vibrios. Their final preparation was lethal in mice when administered by the intraperitoneal route.

We prepared material in a similar fashion from sonicated vibrios and Seitz-filtered stools from cholera patients. The material precipitating between 35 and 55 percent ammonium sulfate was toxic for mice when given intraperitoneally.

The addition or deletion of urea during the preparation as used by Jenkin and Rowley made no apparent change in the final product.

After decanting the supernatant from the last centrifugation, the pellet was dissolved in .01 M sodium phosphate, pH 8.0 in a final volume of 5 ml. per 100 ml. of original material. The protein in this material was fairly constant at approximately 1 mg. per ml.

When 0.1 ml. of this material was added to 20 ml. of Gray's solution bathing the outside of the short-circuited frogskin, there was a very rapid stimulation and then marked depression in the short-circuit current. Fifty percent inhibition of control current occurred within 30 minutes. The stock solution of "toxin" was examined and found to have an osmolality of 900 milliosmols. Only a fraction of this latter value could be accounted for by the .01 M phosphate and the protein content. Estimation of ammonium content gave values as high as 0.8 M.

Addition of 0.1 ml. of 0.8 M $(NH_4)_2SO_4$ or $NH_4Cl$ to the solution bathing the outside of the frogskin gave the same stimulation and inhibition as seen with the "toxin." Addition of 0.1 ml. of 0.8 M $(NA)_2SO_4$ did not affect the frogskin current.

When the "toxin" was dialyzed against a large volume of .01 M phosphate, the effects on the frogskin could no longer be produced, although the protein content was essentially unchanged. After equilibrium dialysis against an equal volume of .01 M phosphate, 0.2 ml. of the outer solution when added to the frogskin had the same effects as $(NH_4)_2SO_4$.

Both the "toxin" preparation and equivalent amounts of ammonium sulfate had pronounced effects on the sodium fluxes of everted intestinal sacs of rabbit ileum (data to be published elsewhere).

It would appear from our experience that, in the preparation of "toxins" by ammonium sulfate fractionation, considerable caution must be exercised to be certain that ammonium itself is not playing a role in the events observed.

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